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January 14, 2009

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Mr. Jay T. Jorgensen, Esq. Sidley Austin LLP 1501 K Street, NW Washington, DC 20005

Re: Missing Considered Materials for Defendant's Expert Dr. Myoda

Dear Jay,

This letter is a follow up to my emails of December 9, 2008, December 19, 2008 and December 30, 2008 where we had identified missing considered materials from Dr. Myoda. You have stated in response to my earlier emails that you would look into these missing documents. Because I have not heard from you and timely receipt of these materials is extremely important, I am writing you again to provide additional details concerning this missing information and reurge your immediate delivery of this important information.

Additionally, as specified below, we also request that you provide us with splits of samples collected by Defendants (or their experts) and splits of the cultures and DNA extracted and evaluated in the labs working with Dr. Myoda and mentioned in Dr. Myoda's Expert Report. Please advise when these samples are being sent and send them to:

Idaho State University
Department of Biological Sciences – MRCF
Attn.: Sid Blackmore
650 Memorial Drive
Pocatello, ID 83209-8007

The contained samples should be clearly marked and identified. They should also be shipped via overnight delivery. Instructions for sample shipment by type are as follows:

For enrichment cultures:

- 1. Grow cultures to late log-phase in enrichment media using appropriate sterile technique.
- 2. Add 50% by volume sterile glycerol for a final volume of approximately 2 milliliters and mix thoroughly.

- 3. Pack glycerol-preserved culture aliquots tightly in a cooler so that they cannot be opened or broken.
- 4. Ship immediately, on ice, and at 4C in coolers overnight express delivery.

For environmental samples including litter, bedding material and environmental samples (i.e. cow hide, goose samples, etc.):

- 1. Provide aliquot of at least 1-5 grams using sterile transfer techniques
- 2. Pack sample aliquots tightly in a cooler so that they cannot be opened or broken.
- 3. Ship immediately, on ice, and at 4C in coolers overnight express delivery.

For DNA extraction:

- 1. Provide aliquot using molecular-appropriate sterile techniques in a total volume of at least 50 microliters in a concentration no less then 10 nanograms of DNA per microliter or if not available at preferred volume and concentration, as much as is available.
- 2. Provide documentation of volume and concentration of DNA provided for each sample.
- 3. Pack sample aliquots tightly in a cooler so that they cannot be opened or broken
- 4. Ship immediately, on dry ice, and at negative 20C in coolers overnight express delivery.

Provide sample identification by directly labeling sampling containers clearly with IDs that can be cross referenced to Myoda's expert Report.

The following materials were not provided in the Myoda considered materials produced by Defendants on December 1, 2008, nor were they provided via a letter to David Riggs dated September 25, 2008 transmitting Myoda-Samadpour 0001-00156.

- 1. <u>Dr. Myoda CV.</u> We still have not received a current CV and a list of his testimony. Please provide current versions of these documents as represented by page 1, Para. 1.1 of his December 1, 2008 Report.
- 2. <u>Missing References.</u> Some references that are cited in the Report text are not listed in the "References" section, nor are they part of the Myoda considered materials. For example, at page 14, para. 7.3 of the Report there is a reference to "Sinton, et.al., (2007) and on page 24, para. 9.2.7 there is a reference to "Hartel et.al., (2007). These reports were not provided as part of the considered materials and no full citation was provided.
- 3. Report Page 27, Para. 9.3.1 The Report states: "In order to demonstrate that IEH had accurately reproduced the methodology, IEH tested pure cultures of *Brevibacterium casei* along with other *Brevibacterium sp.* In addition, IEH evaluated the sensitivity of the assay by increasing the number of cycles."

Please provide the following documents and data relating to the work described in the above quoted statements:

- (a) Documents and data identifying and explaining the methods used to test these cultures including, but not limited to, the PCR mastermix, other materials used in the PCR and the manufacturers of these materials;
- (b) Documents and data identifying and explaining thermocycler conditions;
- (c) Documents and data identifying and explaining DNA cleanup protocols and methods;
- (d) Documents and data identifying and explaining sample handling procedures and methods;
- (e) Documents and data identifying and explaining sampling handling techniques;
- (f) Documents and data identifying the names of the persons who performed this analysis and their training, experience and credentials; and
- (g) Documents and data that provide the details of the QA/QC protocols and methods employed and the results of any QA/QC evaluation for these analyses.

Also, please provide a split sample of each of the cultures tested.

4. Report Page 27, Para. 9.3.2.1 (Part 1). The Report states: "Poultry litter and unused bedding material were supplied to IEH by Defendants. IEH extracted total DNA from both samples and ran the Plaintiff's 'biomarker assays'."

Please provide a split sample of the original samples of the poultry litter and unused bedding and a split sample of the DNA extractions.

- (a) Documents and data identifying and explaining how the DNA was extracted;
- (b) Documents and data identifying and explaining the DNA extraction materials used and the manufacturers of these materials;

- (c) Documents and data identifying and explaining the DNA clean-up protocols and methods;
- (d) Documents and data identifying and explaining the sample handling protocols and methods;
- (e) Documents and data identifying and explaining the aseptic sample handling techniques, protocols and methods;
- (f) Documents and data identifying and explaining the names of the persons who performed this analysis and their training, experience and credentials; and
- (g) Documents and data that provide the details of the QA/QC protocols and methods employed and the results of any QA/QC evaluation for these analyses.
- 5. Report Page 27, Para. 9.3.2.1 (Part 2). The Report states: "The results were that both the PCR and qPCR assays were positive and generated the appropriate products from the poultry litter."

Please provide the following documents and data in conjunction with the above quoted statement:

- (a) The gel images for the PCR results;
- (b) The documents and raw data for the qPCR results, including the CT and melt curve data for all qPCR runs (analysis) performed on these samples (please include appropriate legends/information that allow identification of the qPCR run with the particular samples; i.e., information that allows identification of the isolate qPCR run with a particular sample ID); and
- (c) The documents and data that provide the details of the QA/QC protocols and methods employed and the results of any QA/QC evaluation for these analyses.
- 6. <u>Page 27, Para. 9.3.2.1 (Part 3)</u>. The Report states: "IEH also enriched and incubated the unused bedding material and then extracted total DNA."

Please provide a sample of any incubated materials (bedding materials) as well as (a) a sample of the isolate cultures and (b) a sample of the DNA extracted.

- (a) Documents and data identifying and explaining in detail the protocols and methods used for the incubation;
- (b) Documents and data identifying and explaining the materials used for enrichment (including the manufacturers of such materials);
- (c) Documents and data explaining and identifying the aseptic sample handling techniques;
- (d) Documents and data explaining and identifying sample storage and handling procedures;
- (e) Documents and data identifying the persons who handled the samples and performed the analysis as well as their training, experience and credentials; and
- (f) Documents and data that provide the details of the QA/QC protocols and methods employed and the results of any QA/QC evaluation for these analyses.
- 7. Page 27, Para. 9.3.2.1 (Part 4). The Report states: "The result was that the LA35 PCR primer set generated the appropriate genetic 'signature' from the bedding material."

Please provide the following documents and data in conjunction with the above quoted statement:

- (a) The documents and data identifying and explaining how IEH identified the genetic signature and the methods used by IEH for such identification;
- (b) The documents and data that were used by IEH to identify the genetic signature, including such items as the gel images and the raw data files.
- 8. <u>Page 28, Para. 9.3.2.2 (Part 1)</u>. The Report states: "IEH isolated over 375 cultures from the litter and ran PCR on a subset of those cultures..."

- (a) Documents and data identifying and explaining which samples were used to culture isolates;
- (b) Documents and data identifying and explaining which cultured isolates came from which litter samples;

- (c) Documents and data identifying and explaining the SOPs for the litter sampling, procedures and methods;
- (d) Documents and data identifying and explaining the details of the litter sampling including information on the details of the sampling location, conditions, collection, handling, shipping, receiving, storage and processing of samples prior to culturing;
- (e) Documents and data identifying and explaining the methods for culturing, selecting the subset of the samples for culturing, and analysis;
- (f) Documents and data that identifying and explaining the details of the PCR method used, including the PCR mastermix, other PCR materials used, their manufacturers, the thermocycler conditions, the DNA clean-up protocols, the DNA sample handling procedures, and the aseptic sample handling techniques;
- (g) Documents and data that identify the persons who handled the samples and performed the analysis as well as their experience, training and credentials;
- (h) Documents and data that show the results of the PCR analysis on the subset of analyzed cultures, including the raw data and gel images generated by the analysis;
- (i) Documents and data that show the melt curve for any qPCR performed on these samples, including information that connects (or identifies) these results with a particular litter sample; and
- (j) Documents and data identifying and explaining the QA/QC methods or protocols employed for these analysis and the results of any QA/QC evaluation.

Also, please provide a split sample of the 375 cultures in a viable and culturable state and provide a sample of the DNA extracted from these cultures for the PCR analysis.

9. Page 28, Para. 9.3.2.2 (Part 2). The Report states: "Isolates that gave positive PCR bands of the correct size were then run with qPCR and/or the PCR bands were sequenced. Isolates that generated bands that were not the correct size were set aside."

Please provide split samples of the cultures sent to Molecular Epidemiology, Inc. (MEI) and split samples of the enrichment isolates prepared by MEI and used for the DNA sequencing identification.

Please provide the following documents and data in conjunction with the above quoted statement:

- (a) Documents and data that show the results of the PCR and the qPCR analysis, including the raw data output from the analysis and the gel images;
- (b) Documents and data identifying and explaining the details of the PCR and qPCR methods used including the mastermix, other PCR materials used and their manufacturers, thermocycler conditions, DNA clean-up protocols, sample handling procedures, and aseptic sampling handling techniques;
- (c) Documents and data identifying and explaining the persons who performed the sample handling and the analysis as well as such persons experience, training and qualifications;
- (d) Documents and data showing the CT and melt curve data for any qPCR analysis for a particular isolate run and the sample from which such isolates were obtained;
- (e) Documents and data that provide the details of the QA/QC protocols and methods employed and the results of any QA/QC evaluation for these analyses; and
- (f) Copies of the MEI procedures used to analyze samples and copies of the DNA sequences and the raw data files on the DNA sequences obtained for the submitted samples.
- 10. <u>Page 28, Para. 9.3.2.2 (Part 3)</u>. The Report states: "Isolates that generated the correct size band included strains of: *Brevibacterium avium*, *Brenibacterium epidermidis*, *Corynebacterium Ammoniagenes*, *Pantoea agglomerans*, *Exiguobacterium sp.*, and *lysinbacillus sphaericus*."

- (a) Documents and data identifying and explaining in detail the protocol and methods used to identify these organisms and the documents and data used for such identification (provide both the genetopic and phenotypic identification methods);
- (b) Documents and data identifying and explaining the DNA sequences used for organism identification; and

(c) Documents and data that provide the details of the QA/QC protocols and methods employed and the results of any QA/QC evaluation for these analyses.

Also, please provide a split sample of these isolates in a viable and culturable state as well as a split sample of the DNA extracted from these isolates.

11. <u>Page 31, Para. 9.3.2.3</u>. The Report states: "Three different isolates are represented in FIGURE 9..."

Please provide the following documents and data in conjunction with the above quoted statement:

- (a) Documents and data identifying and explaining the PCR and qPCR method used, including the PCR and qPCR mastermix, PCR and qPCR materials and their manufacturers, thermocycler conditions, DNA cleanup protocols, sample handling procedures, and aseptic sample handling techniques;
- (b) Documents and data that identify the persons handling the samples and performing the analysis as well as documents and data that identify such persons experience, training and credentials;
- (c) Documents and data for any Ct or melt curve for any qPCR analysis performed;
- (d) Documents and data that identify the sample ID with the qPCR analysis and the isolates; and
- (e) Documents and data identifying and explaining the QA/QC procedures employed for such analysis and any QA/QC evaluations of such analysis.

Also, please provide split sample of the cultures analyzed in a viable and culturable state as well as a split sample of the DNA extracted from the isolates that were analyzed.

12. <u>Page 32, Para. 9.3.2.4 (Part 1)</u>. The Report states: "IEH collected and tested 16 faecal samples from Canada Geese in the Seattle, WA area (Green Lake, etc.)."

- (a) Documents and data identifying and explaining the details of the Canada Geese faecal sampling including the sampling protocols, SOPs, and chains of custody, handling, shipping, receiving, storage, processing; and aseptic sample handling procedures;
- (b) Documents and data that identify the persons who did such sampling and processing as well as documents that explain such persons experience, training and credentials; and
- (c) Documents and data that provide the details of the QA/QC protocols and methods employed and the results of any QA/QC evaluation for these analyses.

Also, please provide a split of these Geese samples as well as a split sample of the DNA extracted from such samples.

13. Page 32, Para. 9.3.2.4 (Part 2). The Report states: "The results were that all 16 of the Canada goose samples tested positive for the 'biomarker'."

Please provide the following documents and data related to the above quoted statement:

- (a) Documents and data identifying and explaining the protocols and methods of sample collection, shipping, receiving, storage and processing, including sampling protocols, SOPs, methods, chains of custody, sample locations, and sample type (media);
- (b) Documents and data identifying and explaining the PCR and qPCR method(s) used to test these goose samples, including the PCR and qPCR mastermix, other PCR and qPCR materials, and the manufacturers of materials used, thermocycler conditions, DNA clean-up protocols, sample handling procedures, and aseptic sample handling techniques;
- (c) Documents and data identifying and explaining QA/QC protocols and methods for these analyses as well as any QA/QC findings or report;
- (d) Documents identifying the persons who did such analysis as well as documents that explain such persons training, experience and credentials; and
- (e) Documents and data that provide the details of the QA/QC protocols and methods employed and the results of any QA/QC evaluation for these analyses.

14. <u>Pages 32-33, Para. 9.3.2.5.</u> The Report states: "IEH collected sand samples from Juanita Beach, Kirkland, WA, a swimming beach on Lake Washington... There is no poultry production anywhere near Juanita beach, however, there is a high population of water fowl. The sand samples tested positive for the LA35 biomarker."

Please provide split samples of these samples, a split of the cultures, and a split of the DNA extracted from the cultures.

Please provide the following documents and data related to the above quoted statement:

- (a) Documents and data identifying and explaining the protocols and methods of sample collection, shipping, receiving, storage and processing, including sampling protocols, SOPs, methods, chains of custody, sample locations, and sample type (media);
- (b) Documents and data identifying and explaining the PCR and qPCR method(s) used to test these samples;
- (c) Documents and data identifying and explaining the PCR and qPCR mastermix, (and its manufacturer), the other PCR and qPCR materials used (and their manufacturers), sample handling processes, aseptic sample handling procedures, thermocycler conditions, and DNA clean-up methods and protocols;
- (d) Documents identifying the persons who performed the sampling, shipped the samples, processed the samples and performed the procedures, tests and analysis, as well as documents identifying such persons training, experience and credentials; and
- (e) Documents and data identifying and explaining the QA/QC protocols and procedures as well as any reports or findings concerning the QA/QC for such sampling, processing, and testing.
- 15. Page 33, Para. 9.3.2.6. The Report states: "IEH routinely tests cow/cattle hide samples provided by various slaughter houses for pathogens (E. coli 0157 and salmonella). Some of these samples were tested for the poultry 'biomarker' and were positive. Unfortunately, because these samples tested negative for pathogens and were not collected specifically for this project, they were discarded. Recently, an additional cow hide sample was tested and it too was positive for the poultry 'biomarker'. This sample has been appropriately archived."

Please provide splits of the cultures in a viable and culturable state as well as any samples used and splits of the DNA extracted from these cultures and used for the analysis.

Please provide the following documents and date relevant to the above quoted statement:

- (a) Documents and data identifying and explaining the sampling methods and protocols, including chains of custody, sample location(s), sample state collection, handling, shipping, receiving, storage, processing and aseptic sample handling procedures;
- (b) Documents and data identifying and explaining the PCR and qPCR methods, the PCR and qPCR mastermix (including the mastermix manufacturers), other PCR and qPCR materials (including the manufacturers), thermocycler conditions, and DNA clean-up protocols;
- (c) Documents identifying the persons who collected, shipped and processed the samples and performed the tests and analysis as well as documents and data identifying such persons training, expertise and credentials;
- (d) Documents and data produced during the PCR and the qPCR tests including Ct and melt curve data, and gels;
- (e) Documents and data identifying and explaining which samples correspond with which PCR and qPCR analysis and isolate cultures; and
- (f) Documents and data identifying and explaining the QA/QC methods, procedures and protocols and QA/QC analysis or findings for these procedures.
- 16. Page 33, Para. 9.3.2.7. The Report states: "IEH received 4 samples: B7-crow, C8-dog, B9 and B10-water fowl. The results were that both B9 and B10 were positive for the 'biomarker'."

Please provide splits of these samples as well as splits of these cultures in a viable and culturable state and splits of the DNA extracted from these cultures and used for this analysis.

Please provide the following documents and data relevant to the above quoted statement:

(a) Documents and data identifying and explaining the sampling methods and protocols, including chains of custody, sample location(s), sample state

collection, handling, shipping, receiving, storage, processing and aseptic sample handling procedures;

- (b) Documents and data identifying and explaining the PCR and qPCR methods, the PCR and qPCR mastermix (including the mastermix manufacturers), other PCR and qPCR materials (including the manufacturers), thermocycler conditions, and DNA clean-up protocols;
- (c) Documents identifying the persons who collected, shipped and processed the samples and performed the tests and analysis as well as documents and data identifying such persons training, expertise and credentials;
- (d) Documents and data produced during the PCR and the qPCR tests including Ct and melt curve data, and gels;
- (e) Documents and data identifying and explaining which samples correspond with which PCR and qPCR analysis and isolate cultures; and
- (f) Documents and data identifying and explaining the QA/QC methods, procedures and protocols and QA/QC analysis or findings for these procedures.

Finally, we note that Dr. Samadpor is not listed as a co-author on Dr. Myoda's Report and no considered materials were submitted on behalf of Dr. Samadpor. Is Dr. Samadpor a retained testifying expert witness for the Defendants? If so, where are his Report and considered materials? Please state clearly his status as an expert for the Defendants.

Since this information is now over 6 weeks past due we anticipate a prompt response and production of this information and the split samples.

Sincerely,

David P. Page

DPP/sdk

cc: Plaintiff's counsel and Defendants' counsel